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Evaluation of maternal serum galectin-9 levels in pregnancies with the threat of preterm birth

Savaş Özdemir[1], Fatih Şahin[1], Gül Özel Doğan[2], Özlem Baytekin[3]
[1] Department of Obstetrics and Gynecology, Prof. Dr. Cemil Taşçıoğlu City Hospital
[2] Department of Obstetrics and Gynecology, Şişli Hamidiye Etfal Hospital
[3] Department of Biochemistry, Şişli Hamidiye Etfal Hospital, Istanbul, Turkey

Abstract
Aim: Galectins expressed in various locations in the maternal-fetal interface play a role primarily in immunomodulation, embryo implantation, trophoblast invasion, and angiogenesis, which are early pregnancy events. Therefore, they are responsible for the physiological and healthy course of pregnancy. We aimed to evaluate the usability of the Galectin-9 (Gal-9) marker in predicting the risk of preterm birth.
Material and Methods: This prospective study included 40 singleton pregnancies (20-37 weeks) with threatened preterm labor and 40 healthy pregnancies without obstetric complications. The maternal Galectin-9 levels in serum were measured.
Results: No statistically significant difference was found in terms of maternal Galectin-9 levels between the preterm labor and control groups (p=0.758). In pregnancies with threatened preterm labor, white blood cell (WBC) levels were found to be statistically lower than in the control group (p=0.046). There was a significant positive correlation between systolic blood pressure level and WBC level (p=0.015). A statistically significant negative correlation was found between the WBC level and the Galectin-9 level (p=0.007).
Discussion: There is no relationship between maternal Galectin-9 level and threatened preterm labor in complicated pregnancies.

Keywords
Galectin-9, Preterm Labor, Immune Modulation

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Corresponding Author: Fatih Şahin, Department of Obstetrics and Gynecology, Prof. Dr. Cemil Taşçıoğlu City Hospital, Sisli, Istanbul, Turkey.
E-mail: fatih_sahin67@hotmail.com P: +90 544 743 01 67
Corresponding Author ORCID ID: https://orcid.org/0000-0002-1621-5896
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Introduction

Preterm birth remains an unresolved health problem, accounting for about 10% of live births worldwide. Due to vulnerable body surfaces, immature immunity, and a variety of injurious exposures, preterm infants are predisposed to sepsis particularly in the first 4 weeks of life. This is a continuous challenge to clinicians who are involved in the care of preterm infants, since sepsis and persistent inflammation are considered crucial mediators for mortality and the development of long-term morbidities [1]. Therefore, there is an urgent need to identify risk factors and biomarkers aiming at the prevention and early treatment of sepsis.

Galectins are a family of beta-galactoside-binding proteins that are non-classically secreted and have recently received considerable attention in the spatio-temporal regulation of surface 'signal lattice' organization, membrane dynamics, cell-adhesion and disease therapeutics. Galectin-9 is a unique member of this family, with two non-homologous carbohydrate recognition domains connected by a linker peptide sequence of variable lengths, resulting in the generation of isoforms with distinct properties and functions in both physiological and pathological settings, such as during development, immune reactions, neoplastic transformations and metastasis [2]. Galectins, a protein group commonly found in mammals, regulate various fundamental biological processes [3]. Until today, 13 subtypes have been identified in humans [4]. The biological functions vary among subtypes depending on the presence of a suitable ligand and even local concentrations, thus creating functional diversity [5]. Galectins expressed in various locations in the materno-fetal region are mainly involved in early pregnancy events such as immune modulation, embryonic implantation, trophoblast invasion, and angiogenesis [6]. Therefore, they are responsible for the physiological and healthy course of pregnancy. Due to their immunomodulatory functions, galectins act as a shield preventing the rejection of the semi-allogeneic fetus, and as a result, they also contribute to the continuation of pregnancy by preventing its termination. Although galectin interactions during gestation are well characterized, potential outcomes of these interactions in the context of preterm birth are poorly defined.

In this study, we aimed to evaluate the usability of the Galectin-9 biomarker in predicting the risk of preterm labor.

Material and Methods

The study was carried out with the permission of 'Istanbul Health Sciences University Şişli Hamidiye Etfal Training and Research Hospital' Clinical Research Ethics Committee (Date: 11.10.2021, Decision No: 354). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This prospective study included pregnant women between the ages of 18-40 years who were admitted with indications of preterm labor between November 1, 2021 and May 1, 2022 at the Istanbul Health Sciences University Şişli Hamidiye Etfal Training and Research Hospital Obstetrics and Gynecology Clinic. The study also included a control group of healthy pregnant women who presented for routine clinic visits and had no uterine contractions. Pregnant women with membrane rupture, hypertension, multiple pregnancies, diabetes, thyroid diseases, chronic diseases, body mass index over 30 kg/m2, preeclampsia, intrauterine growth retardation, or fetal anomalies were not included in the study. Preterm labor is defined as regular uterine contractions accompanied by cervical dilation (>3 cm) or cervical length of less than 20 mm between 20 weeks and 36 weeks 6 days of gestation, based on the last menstrual period and ultrasound findings. Pregnant women diagnosed with preterm labor were admitted to the hospital. IV fluid therapy was administered, and routine laboratory tests, including complete blood count, biochemistry, urinalysis, coagulometer, urine culture, cervical culture, and vaginal culture tests were performed. Women with a gestational age of 34 weeks or less were given tocolytic and corticosteroid therapy. Informed written consent was obtained from pregnant women admitted with preterm labor for whom we collected blood samples to investigate the Galectin-9 biomarker, in addition to routine tests. Each patient provided a onetime 10 mL sample of the whole blood collected into a K2EDTA tube for further testing. Each sample was centrifuged for 10 min at 2500 rpm and the collected serum was stored at −80 °C until analysis. The serum concentration of galectin-9 was assayed in duplicate using an enzyme-linked immunoassay (ELISA) kit according to the manufacturer's protocol. All patient records were recorded on case report forms.

Statistical Analysis

A power analysis was performed in advance using G*Power version 3.1.9.7 to determine the minimum sample size required to test the study hypothesis [7]. Results indicated that the required sample size to achieve 80% power for detecting a medium effect, at a significance criterion of $\alpha = .05$, was N = 80 for [One way ANOVA]. SPSS 15.0 for Windows program was used for statistical analysis. Descriptive statistics were presented as numbers and percentages for categorical variables and as mean, standard deviation, minimum, maximum, and median for numerical variables. Ratios in independent groups were compared with the Chi-square test. Comparisons of numerical variables in independent two groups were performed with the Student t-test when the normal distribution condition was met, and with the Mann-Whitney U test when the condition was not met. Relationships between numerical variables were examined using Spearman's Correlation Analysis as the parametric test condition was not met. The statistical alpha significance level was accepted as $p<0.05$.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

The mean parity of patients with non-stress test (NST +) who are at risk of preterm birth was found statistically lower compared to the control group (NST -) ($p=0.046$). Cervical length (CX) was found significantly lower in pregnant women at risk of preterm birth ($p<0.001$). The mean white blood cell (WBC) count in NST + pregnant women was found statistically lower compared to the control group (NST -) ($p=0.046$). Fever was statistically higher in pregnant women at risk of preterm birth compared to the control group ($p=0.042$). Diastolic blood pressure was found significantly higher in NST + pregnant

women (p=0.005). The mean hemoglobin (HB) levels in NST + pregnant women were found statistically higher compared to the control group (p=0.049).

There was no statistically significant difference between the mean Galectin-9 levels of the preterm birth at risk and control groups (p=0.758). The findings are summarized in Table 1.

Culture results, antibiotic, tocolytic, and corticosteroid usage in patients at risk of preterm birth with a positive non-stress test (NST +) are summarized in Table 2.

A significant positive correlation was found between Galectin-9 levels and systolic blood pressure (p=0.015). A significant negative correlation was found between white blood cell (WBC) levels and Galectin-9 (p=0.007). The findings are summarized in Table 3.

Table 1. Demographic and laboratory data of patients.

		NST		p
		Positive	Negative (control)	
		SD Median (Min-Max)	Mean.±SD (Min-Max)	
Age		27,2±6,4	27,6±5,5	0,779*
		26,5 (16-44)	27,5 (18-40)	
Gravida		1,88±1,64	1,95±1,18	0,193
		1 (1-8)	2 (1-6)	
Parity		0,40±0,81	0,75±1,06	0,046
		0 (0-4)	0,5 (0-5)	
Abortus		0,48±1,20	0,20±0,52	0,467
		0 (0-6)	0 (0-2)	
LMP (weeks)		30,8±3,2	31,2±3,5	0,421
		31 (23-35)	32 (23-35)	
		3,0±2,1	3,6±2,0	0,209
		3 (0-6)	4 (0-6)	
LMP	≤ 27 w	7 (17,5%)	8 (20,0%)	0,775
	> 27 w	33 (82,5%)	32 (80,0%)	
Body temperature		36,0±0,1	36,0±0,0	0,042
		36 (36-36,6)	36 (36-36)	
Pulse		76,7±8,8	73,3±7,0	0,093
		75 (62-92)	71,5 (62-88)	
Systolic Blood Pressure		112,5±7,3	114,6±6,4	0,197
		112 (90-121)	113,5 (95-130)	
Diastolic Blood Pressure		69,8±7,0	65,9±5,9	0,005
		70 (51-82)	65 (60-86)	
Bishop Score		2,63±2,29	0,0±0,0	<0,001
		2,5 (0-8)	0 (0-0)	
CX (mm)		25,2±8,2	35,1±5,4	<0,001
		25 (6-40)	35,5 (24-45)	
HB		11,5±1,3	10,9±1,4	0,049*
		11,55 (8,8-14,9)	10,9 (7,8-13,1)	
HTC		33,9±3,6	32,8±3,6	0,147*
		34 (26-43)	33 (26-38)	
WBC		10,9±4,2	12,1±3,9	0,046
		9 (6-23)	11 (7-26)	
GALECTIN-9 pg/mL		314,5±184,4	483,1±593,9	0,758
		259,8 (113,5-899,6)	243,2 (65,85-3025)	

*Student t-test #Mann-Whitney U Test, NST: non-stress test HTC: hematocrit LMP: last menstrual period CX: cervix WBC: white blood cell HB: hemoglobin

Table 2. Culture results, antibiotic, tocolytic, and corticosteroid usage.

		NST Positive	
		n	%
Urinary Culture Result	No Growth	37	92,50%
	Positive Growth	3	7,50%
	Enterococcus Faecalis	1	2,50%
	Klebsiella Pneumoniae	2	5,00%
Cervical Culture Result	No Growth	39	97,50%
	Positive Growth	1	2,50%
	Group B Streptococcus	1	2,50%
Vaginal Culture Result	No Growth	38	95,00%
	Positive Growth	2	5,00%
	Candida Albicans	2	5,00%
Antibiotic	No Growth	36	90,00%
	Positive Growth	4	10,00%
Antibiotic	Ampicillin ®	2	5,00%
	Cefazolin ®	1	2,50%
	Nitrofurantoin ®	1	2,50%
Tocolytic	Not Given	8	20,00%
	Nifedipine ®	18	45,00%
	Nifedipine ® + Indomethacin ®	13	32,50%
	Indomethacin L®	1	2,50%
Corticosteroid	Negative	5	12,50%
	Positive	35	87,50%

Table 3. Spearman's Correlation Analysis.

	Galectin-9 pg/mL	
	R	p
Age	-0,116	0,305
Gravida	-0,062	0,587
Parity	-0,045	0,692
Abortus	-0,013	0,911
LMP (weeks)	0,201	0,074
Systolic Blood Pressure	0,272	0,015
Diastolic Blood Pressure	0,121	0,286
Bishop Score	-0,076	0,502
CX (mm)	-0,008	0,942
HGB	-0,071	0,529
HTC	-0,088	0,436
WBC	-0,297	0,007

HTC: hematocrit LMP: last menstrual period CX: cervix WBC: white blood cell HB: hemoglobin

Discussion

The aim of this study was to investigate the maternal serum levels of galectin-9 in pregnancies at risk of preterm birth. There are studies in the literature on galectin and adverse pregnancy outcomes; in a study conducted, Galectin-1 (Gal-1) levels were found to be low in pregnant women with preterm prelabor rupture of membranes (pPROM), but no significant relationship was observed with maternal serum levels of Galectin-9 [8].

Until now, little has been known about Galectin-9 (Gal-9), and there are limited data on its full function and properties. Galectin-9 is expressed not only by the endometrium, but also by trophoblasts, stromal cells in the decidua, endothelial cells of

the placenta, and several types of immune cells [9,10]. Similar to Gal-1, in a murine model, Gal-9 participates in processes that are involved in the local anti-inflammatory environment, enabling implantation and early fetal development [11]. Additionally, Gal-9 is responsible for the suppression of uterine natural killer (NK) cells via secretion in endometrial stromal cells [9]. However, although a similar role for Gal-9 has been proposed in human pregnancy, the available data are based only on a mouse model [8]. As pregnancy progresses, the expression and concentration of Gal-9 in the mother's blood increase, making it another galectin that is likely to be very important in maintaining pregnancy [12]. Regarding the available data on pregnancy complications, several studies have found an association between low Gal-9 expression and spontaneous miscarriages [9]. Similarly, there are studies linking low Gal-9 and recurrent pregnancy loss [13]. It has been suggested that the abnormal Tim-3/Gal-9 pathway, due to the immunomodulatory effect of Gal-9, may contribute to preeclampsia [14]. Interestingly, some studies have shown that serum levels of Gal-9 are higher in women carrying a male fetus compared to a female fetus [15]. In our study, there was no significant difference in Gal-9 levels between pregnant women at risk of premature birth and the control group.

The limitations of this study include a small number of participants and being conducted at a single center. Additionally, examination of the placenta and fetal membranes may provide value for future studies. However, to investigate Gal-9 levels as potential, promising clinical markers for the prediction of preterm labor, further clinical studies on larger groups are required.

Conclusion

There is no relationship between maternal galectin-9 levels and preterm labor risk in pregnant women.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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